



Trilogy Laboratory Fluorometer

User's Manual

Version 1.1
August 01, 2006
P/N 998-7210

Turner Designs

845 W. Maude Ave.
Sunnyvale, CA 94085
Phone: (408) 749-0994
FAX: (408) 749-0998
www.turnerdesigns.com

This Page Intentionally Left Blank

Highlights of the Trilogy Fluorometer

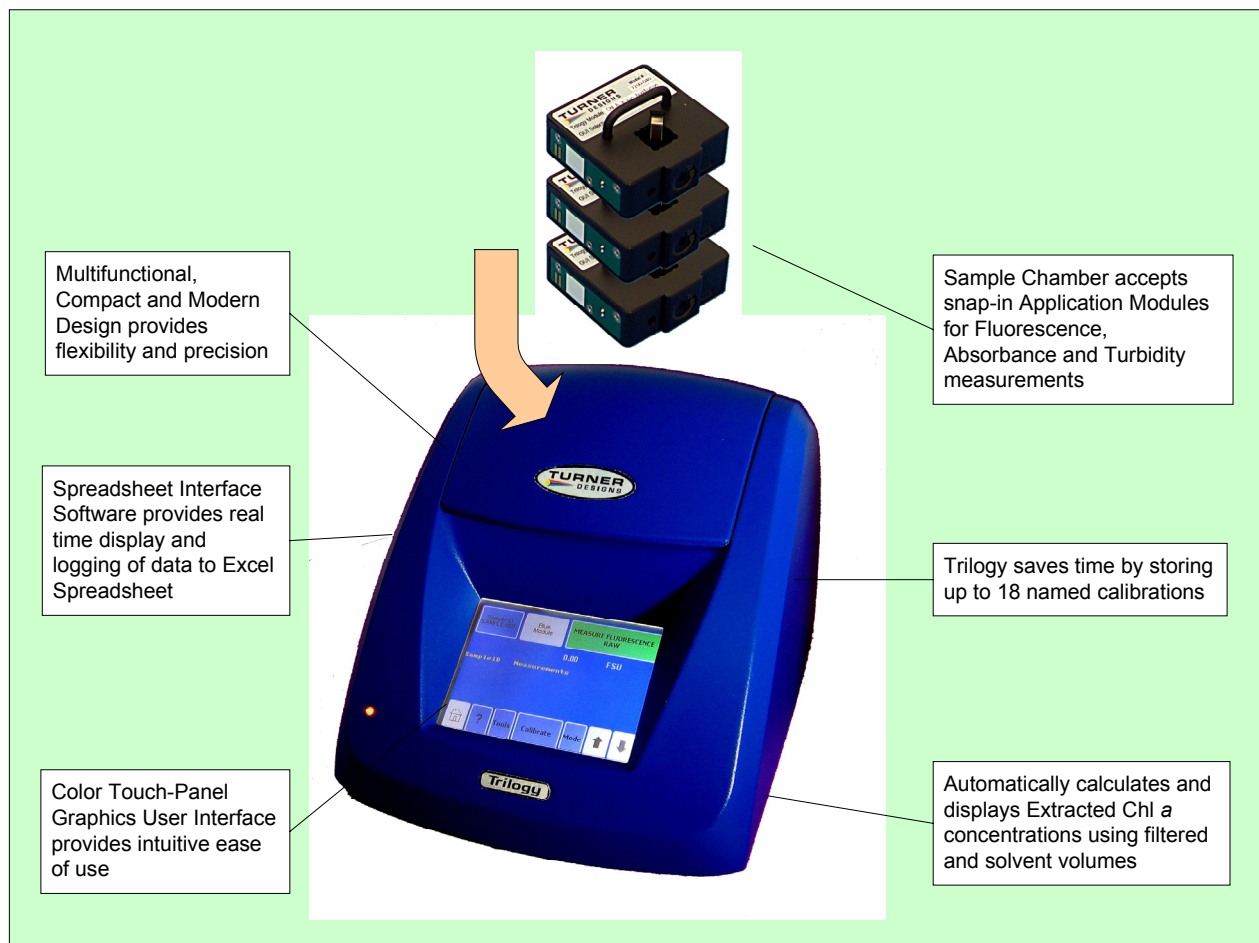


Table of Contents

CHAPTER	DESCRIPTION	PAGE
	<i>Highlights of the Trilogy Fluorometer</i>	<i>3</i>
	<i>I. Getting Started</i>	<i>5</i>
	Description	5
	Unpacking and Inspection	5
	Setup	6
	Getting to the Home Screen	6
	Precautions	7
	<i>II. Fluorometer Operation</i>	<i>8</i>
	Touch screen Basics (Fluorescence)	8
	Measuring Samples	9
	<i>III. Absorbance Operation</i>	<i>11</i>
	Touch Screen Basics (Absorbance)	11
	Measuring Samples	12
	<i>IV. Turbidity Operation</i>	<i>14</i>
	Touch Screen Basics (Turbidity)	15
	Measuring Samples	15
	<i>V. Calibration Overview</i>	<i>17</i>
	A. Why Calibrate	17
	B. When to Calibrate	17
	C. Trilogy Calibration Options, (Fluorescence and Turbidity)	17
	D. Trilogy Calibration Procedures	17
	E. “Direct Calibration” Procedure – a) Fluorescence and Turbidity Modules, Single Point and Multi Point Cal	18
	F. Extracted Chlorophyll Measurements with the Chl <i>a</i> – Acidification Fluorescent Module	20
	G. Calibrating and Displaying Corrected Readings for the Chl <i>a</i> Acidification and Non-Acidification Modules	20
	H. “Direct Calibration” Procedure – b) Extraction, Acidification, Single Point and Multi Point	20
	I. “Direct Calibration” Procedure – c) Extraction, Non-Acidification, Single Point and Multi Point	23
	J. Using the Secondary Standard	23
	K. Use of the Solid Secondary Standard for Chlorophyll Applications	23
	L. Use of the Solid Secondary Standard for Dye Applications	24
	M. Trilogy Calibration Procedure, (Absorbance Module)	24
	<i>VI. Touch Screen Basics</i>	<i>26</i>
	Touch Screen	26
	<i>VII. Systems Connections</i>	<i>27</i>
	Serial Output Functional Check	27
	<i>VIII. Spreadsheet Interface Software Installation</i>	<i>28</i>
	Excel Spreadsheet Example	28
	<i>Appendix A - Troubleshooting</i>	<i>29</i>
	Fluorescence Troubleshooting	29
	Absorbance Troubleshooting	29
	Turbidity Troubleshooting	30
	Spreadsheet Interface Software Troubleshooting	30
	<i>Appendix B - Warranty and Obtaining Service</i>	<i>32</i>
	Warranty	32
	Out of Warranty Service	32
	<i>Appendix C – Specifications</i>	<i>33</i>
	<i>Appendix D – Principles of Fluorescence</i>	<i>34</i>
	Principles of Fluorescence	34
	<i>Appendix E – Recommended Measurement Practices</i>	<i>35</i>
	Linear Range and Quenching	35
	Temperature Considerations	35
	<i>Appendix F - Chlorophyll <i>a</i> Acidification and Non-Acidification Calculations</i>	<i>36</i>
	<i>Appendix G - In Vivo Fluorescence Measurements Using Trilogy</i>	<i>37</i>

I. Getting Started

Description

The Trilogy Laboratory Fluorometer is a compact, multifunctional laboratory instrument that can be used for making fluorescence, absorbance and turbidity measurements using the appropriate snap-in Optical Module. A color touch screen with simple menus makes for an intuitive user interface.

Fluorescence Modules are available for discrete sample measurement of various fluorescent materials including chlorophyll (*in vivo* and extracted), Rhodamine, fluorescein, cyanobacteria pigments, ammonium, CDOM, optical brighteners and other fluorescent compounds.

The Absorbance Module accepts interchangeable filter paddles so measurements can be made at different wavelengths in order to identify or place a sample in a particular class of compounds. The standard filter paddle wavelengths/bandwidths are: 560/10; 600/10 and 750/10 nm.

The Turbidity Module uses an Infrared (IR) LED with a wavelength of 850 nm as required for reference method: ISO 7027/DIN EN 27027, "Water Quality – Determination of Turbidity". Using Infrared allows Turbidity to be measured at wavelengths that are not normally absorbed by organic matter thereby reducing susceptibility to interference by sample color.

When properly calibrated, the Trilogy Fluorometer will read out the actual concentration of the solution. Optical Modules contain the necessary light source and filters for the relevant application

Unpacking and Inspection

Upon receiving the Trilogy, please inspect it carefully and make certain all accessories are present. Refer to the checklist shipped with the instrument for order-specific items.

A typical Trilogy shipment includes:

- Checklist
- Trilogy Laboratory Fluorometer
- CD – Trilogy, includes files for: User's Manual (pdf); Quick Start Guide (pdf); Spreadsheet Interface Software (pdf)
- Solid Secondary Standard, (not intended for use with Ammonium optical module)
- Power Supply Kit
- RS-232 Cable
- Quick Start Guide, (hardcopy)
- 10 x 10 mm Methacrylate Cuvettes, (quantity of 10)
- 12 x 35 mm Glass Round Vial, (quantity of 5)
- 12 mm Round Adaptor
- 50-Well Microtube Storage Box
- Optical Module(s) as ordered
 - Fluorescence Module
 - Absorbance Module
 - Turbidity Module

Setup

Place the Trilogy Fluorometer on a flat, level surface. Allow at least 6 inches (16 cm) of clearance above the instrument to open and close the lid. Position the instrument so that the touch screen faces you.

Power Supply

Connect the power supply into the power connection of the instrument, see Figure 1, and plug it into a wall outlet. See Specifications for power requirements.

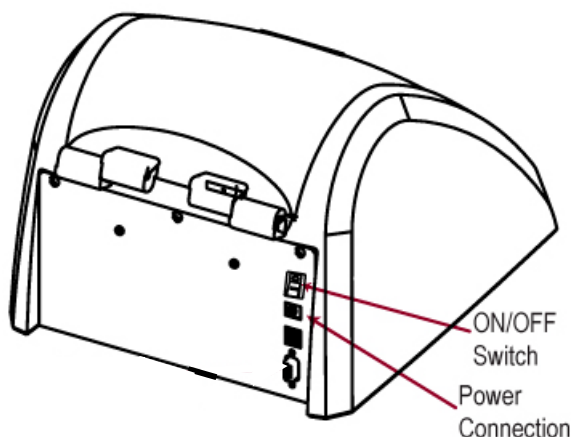


Figure 1.

Getting to the Home Screen

1. With the unit turned off, insert an optical module into the base for the sample adapter, (see Fig 2). **Be sure to close the fluorometer lid so that the Trilogy power on step will complete successfully.**
2. Turn on the On/Off switch located on the back of the Trilogy, see Figure 1. Verify the display becomes active, and shows the module selection screen; see Figure 3 – Module Selection.

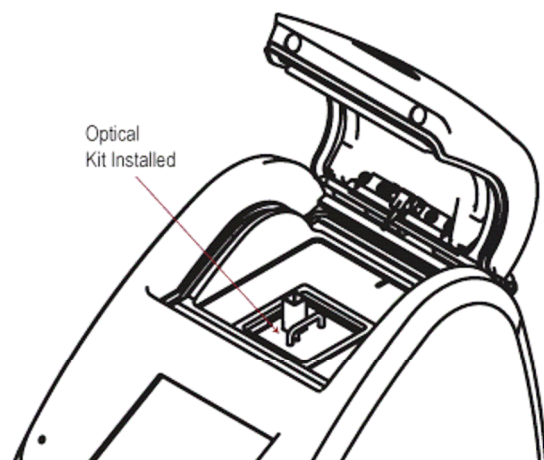


Figure 2 showing Optical Kit installed in preparation for getting to the Home Screen.

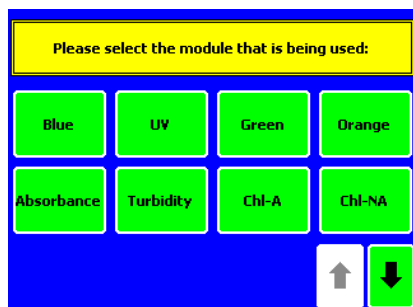


Figure 3 Module Selection Screen

3. Select the module inserted, and touch “OK” on the confirming screen.
4. When the display indicates the Home Screen, you are now ready to use Trilogy in its Raw Mode, (measurements are relative), or calibrate Trilogy and its associated snap-in Application Module to make quantitative measurements.

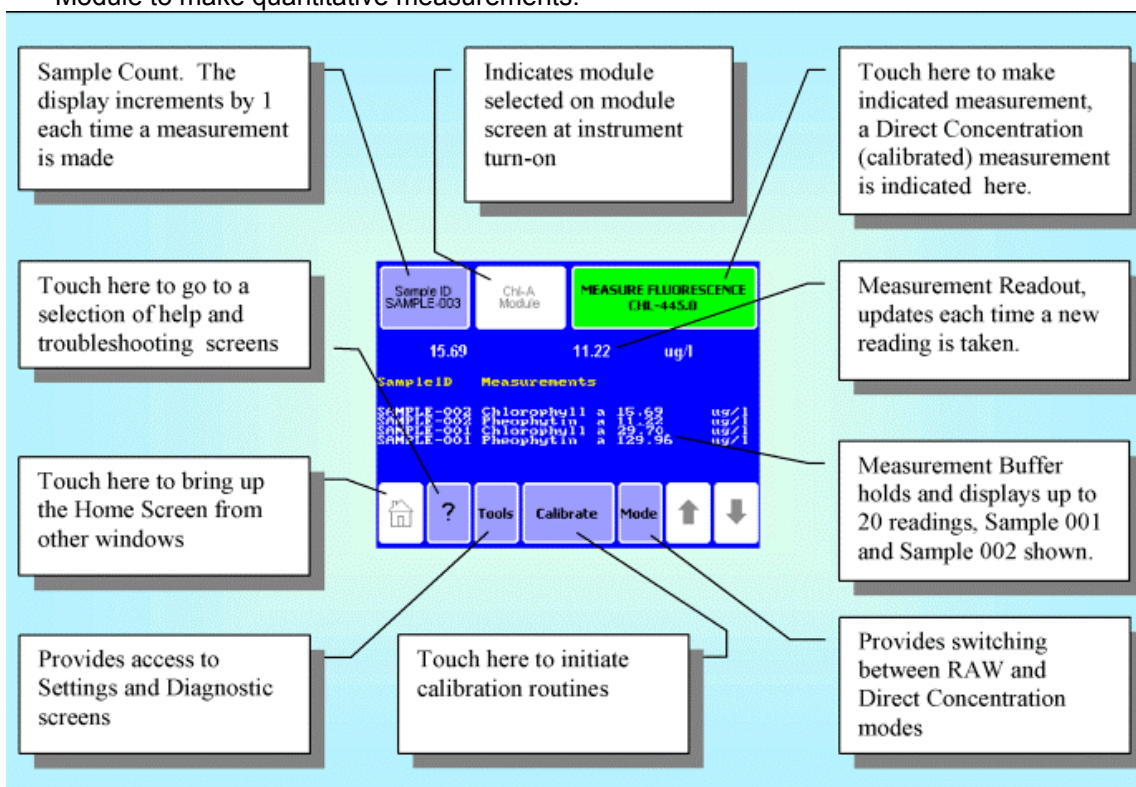


Figure 4: Trilogy Home Screen Display, (Fluorescence Module In Use)

Precautions

The Trilogy is intended for indoor use only. Wipe up spills immediately. The Trilogy contains sensitive optical components and precision-aligned mechanical assemblies. Avoid rough handling. Do not leave the lid open for extended periods of time. Turn OFF the Trilogy to change Optical Application Modules, or to install a different module.

Note: After 20 minutes without activity or user stimulation, the Touch Screen hibernates. Lightly touch the screen to activate.

II. Fluorometer Operation

Fluorescence Optical Module

Installation

1. Power the Trilogy OFF
2. Grasp the handle of the Optical Application Module and align the kit with the sample compartment.
3. Press down firmly to lock the Optical Application Module in place. (See Figure 5.)
4. Close the lid and power ON the Trilogy. Use the touch screen to identify the type of Optical Application Module installed.

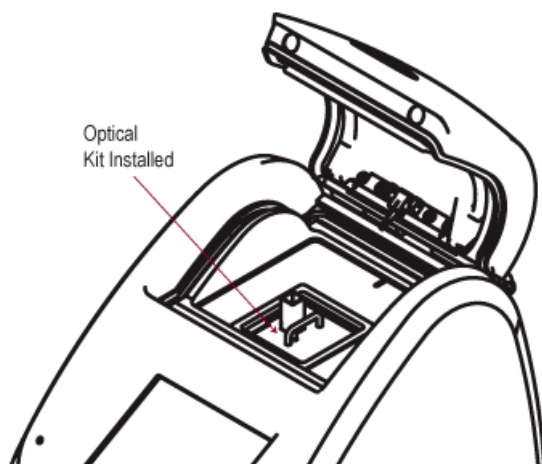


Figure 5.

Removal

1. Power OFF the Trilogy before removing the Optical Application Module
2. Grasp the handle and gently pull up to release the kit from the sample compartment.

Touch screen Basics (Fluorescence)

Home Screen

The "Home" screen appears after confirmation of the Optical Application Module. The "Home" screen provides orientation for the multiple functions of the Trilogy. From the "Home" screen, select "Calibrate," "Tools," "Mode," and "Help." The "Home" screen is also the measurement screen. (See over for Figure 6 showing Home Screen with callouts).

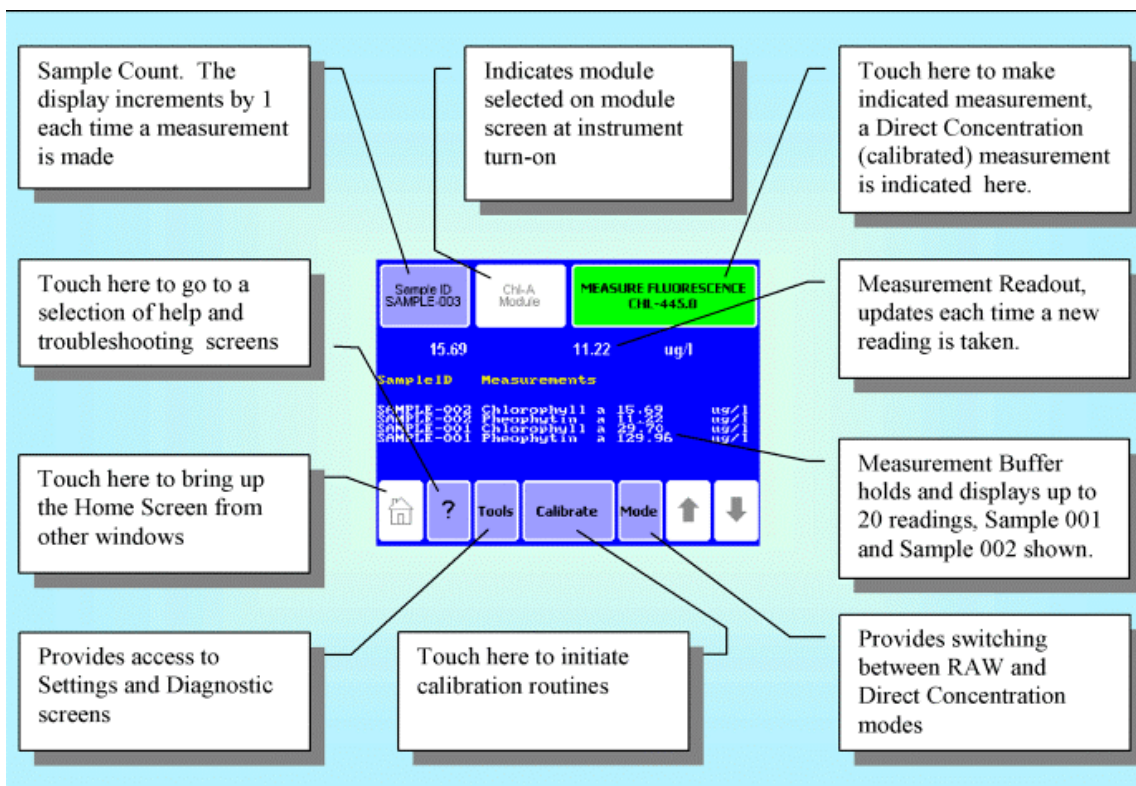


Figure 6 – Trilogy Home Screen for Fluorescence Module Configuration.

Calibration

Refer to Section V.

Measuring Samples

There are two measurement modes available on the Trilogy when using the Fluorescence Module:

- Raw Fluorescence Mode – No calibration required
- Direct Concentration Mode – Calibration required – see Section V

Touch **"Mode"** on the Home Screen to select the measurement mode.

- 1. Raw Fluorescence Mode:** The Raw Fluorescence Mode should be used for qualitative measurement, for example where measuring changes is required, rather than absolute concentration values. Readings are displayed in Relative Fluorescence Units (RFU).
- 2. Direct Concentration Mode:** The Direct Concentration mode makes absolute measurements based on a calibration, see Section V for the Calibration Procedure.

The Trilogy accommodates 10 x 10 mm methacrylate and polystyrene cuvettes (minimum 2 mL volume). Use 12 mm x 35 mm glass test tubes for extracted chlorophyll measurements, and use methacrylate for ammonium measurements

1. Open the lid of the Trilogy and insert the cuvette. Close the lid.

2. Touch "Sample ID" to name your sample (optional).
3. Using the keypad, enter the sample name into the name field. Touch "Save" to save the sample ID.
4. Touch "Measure Fluorescence" to commence measurement. The Trilogy will measure the sample for 6 seconds and report the average reading for the sample.

The Trilogy reports data on the "Home" screen and displays the results for the most recent 20 measurements. Use the arrow keys to scroll through the most recent measurements. The data automatically exports to a printer or PC when properly connected, (see Section VII). Please note the Trilogy does not store more than 20 measurements at one time. If more than 20 readings are taken, the oldest reading will be overwritten. Measurements are not stored between power cycles.

Tools (Fluorescence)

Touch the "Tools" key to access "Settings."

Tools - Settings

View Cal Details

Touch "View Cal Details" to see information on the current calibration for Direct Concentration Mode. "View Cal Details" specifically provides information on the raw fluorescence for each standard and the blank as well as the unit of measure and the Optical Application Module.

Continuous Sampling

The Continuous Sampling feature enables repeat measurements at user-defined intervals.

1. Touch "Continuous Sampling" and turn the feature ON. Highlight the frequency of measurement and the number of total measurements. The maximum number of total measurements is 9999.
2. Touch "OK" to return to the "Home" screen.
3. Connect the Trilogy to a printer or a PC to collect the data. Touching the screen repeatedly causes an early-abort of Continuous Sampling measurements.

Measurement Tip:

On the Home Screen, touch **Settings** then touch the **Lid Start** key to turn the feature ON. When the Lid Start feature is ON, measurement begins as soon as the lid closes. The lid start feature allows for immediate measurement and eliminates the need to touch the "Measure" key. Also, the touch screen does not hibernate when Lid Start is ON.

Return to the **Lid Start** key under the **Settings** menu to turn the feature OFF.

III. Absorbance Operation

Absorbance Module

Installation

1. Power OFF the Trilogy.
2. Align the Absorbance Module with the sample compartment.
3. Press down to lock the Absorbance Module in place.
4. Close the lid and power ON the Trilogy. Select "Absorbance" from the list of options on the touch screen.
5. Install the filter paddle that corresponds to the wavelength of absorbance for the assay. (See Figure 7.)

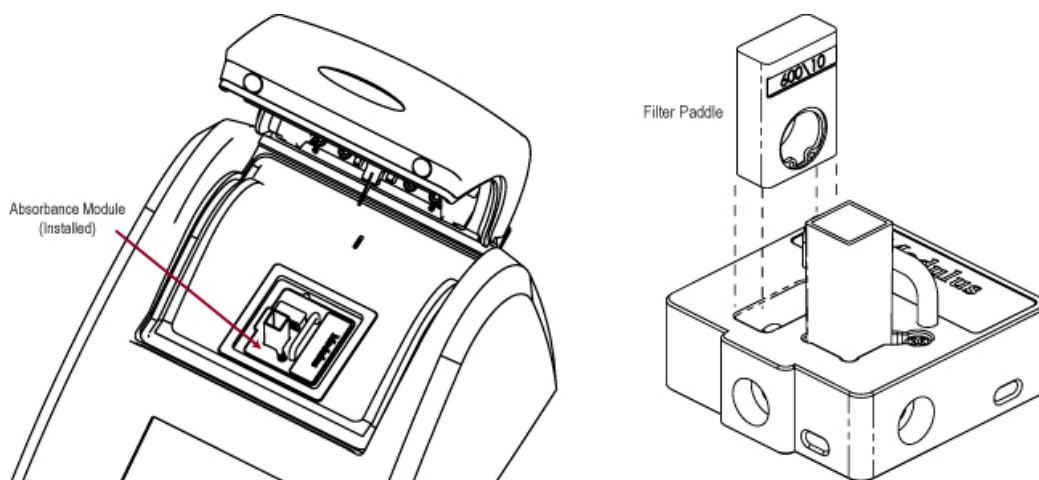


Figure 7.

Removal

1. Power OFF the Trilogy before removing the Absorbance Module.
2. Grasp the handle and gently pull up to release it from the sample compartment.
3. Close the lid to protect the light detector from exposure to ambient light. Alternatively, install an Optical Application Module.

Touch Screen Basics (Absorbance)

Home Screen

The "Home" screen appears after confirming the photometer operation by installing the Absorbance Module. From the "Home" screen, select "Calibrate," "Tools," "Mode" and "Help." The "Home" screen is also the measurement screen, see Figure 8 on next page.

Mode

Touch "Mode" to select the unit of measure for absorbance. The available options include Absorbance units (Ab) and Transmittance (%T).

The following formulas describe the method of the Trilogy Absorbance Module for measuring absorbance:

$$Ab = \text{Lg}[(b-z)/(s-z)]$$

$$\%T = [(s-z)/(b-z)] * 100$$

where

z=zero

b=baseline

s=signal

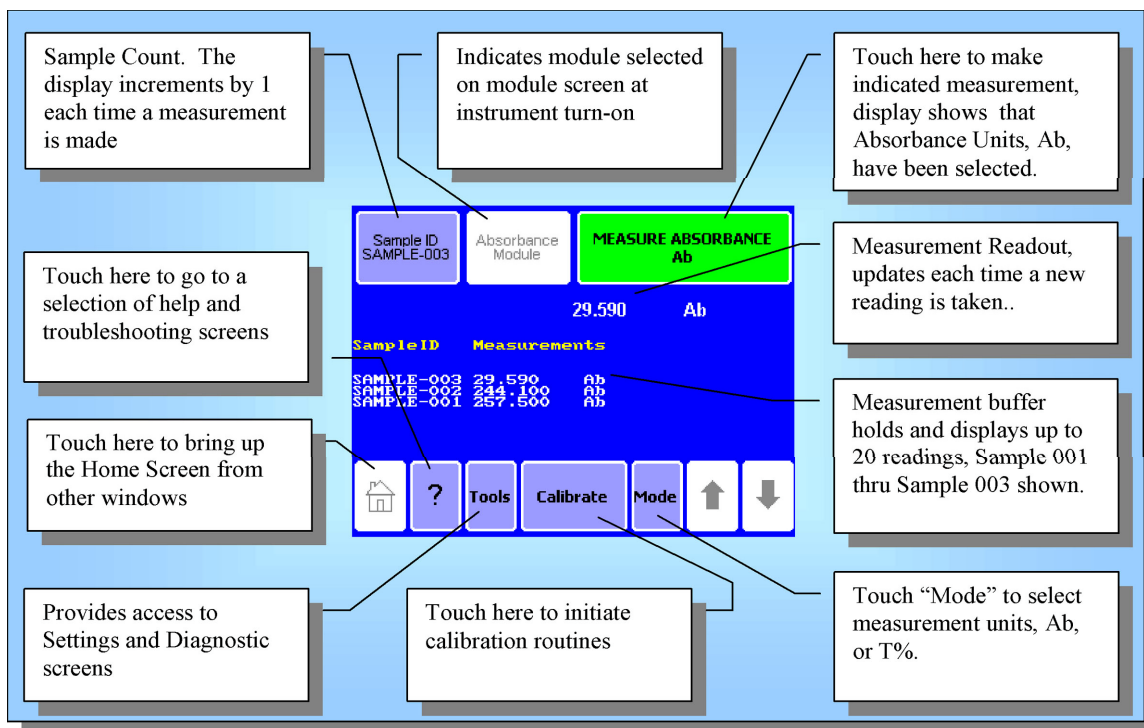


Figure 8: Home Screen When Using Absorbance Module

Calibration

See Section V.

Measuring Samples

The Absorbance Module accommodates 10 x 10 mm methacrylate and polystyrene cuvettes as well as glass cuvettes (minimum 1.5 mL volume).

1. Open the lid and insert the cuvette. Close the lid.
2. Touch "Sample ID" to name your sample (optional). Using the keypad, enter the sample name into the name field. Touch "Save" to save the sample ID.
3. Touch "Measure Absorbance" to commence measurement. The Trilogy will measure the sample for 6 seconds and report the average reading for the sample.

The Trilogy reports data on the "Home" screen and displays the results for the most recent 20 measurements. Use the arrow keys to scroll through the most recent measurements. The data exports to a printer or PC if properly connected, (see Section VII).

Please note the Trilogy does not store more than 20 measurements at one time. If more than 20 readings are taken, the oldest reading will be overwritten. Measurements are not stored between power cycles.

Tools - Settings

Touch the "Tools" key to access "Settings."

View Cal Details

Touch "View Cal Details" to see information on the current calibration for the baseline and the zero.

Continuous Sampling

The Continuous Sampling feature enables repeat measurements at user-defined intervals.

1. Touch "Continuous Sampling" and turn the feature ON.
2. Highlight the frequency of measurement and the number of total measurements. The maximum number of total measurements is 9999.
3. Touch "OK" to return to the "Home" screen.
4. Connect the Trilogy to a printer or a PC to collect the data obtained during Continuous Sampling. Touching the screen repeatedly causes an early-abort of Continuous Sampling measurements.

Measurement Tip:

On the Home Screen, touch **Settings** then touch the **Lid Start** key to turn the feature ON. When the Lid Start feature is ON, measurement begins as soon as the lid closes. The lid start feature allows for immediate measurement and eliminates the need to touch the "Measure" key. Also, the touch screen does not hibernate when Lid Start is ON.

Return to the **Lid Start** key under the **Settings** menu to turn the feature OFF.

IV. Turbidity Operation

Turbidity Optical Module

Installation

1. Power the Trilogy OFF
2. Grasp the handle of the Optical Application Module and align the kit with the sample compartment.
3. Press down firmly to lock the Optical Application Module in place. (See Figure 9.)
4. Close the lid and power ON the Trilogy. Use the touch screen to identify the type of Optical Application Module installed.

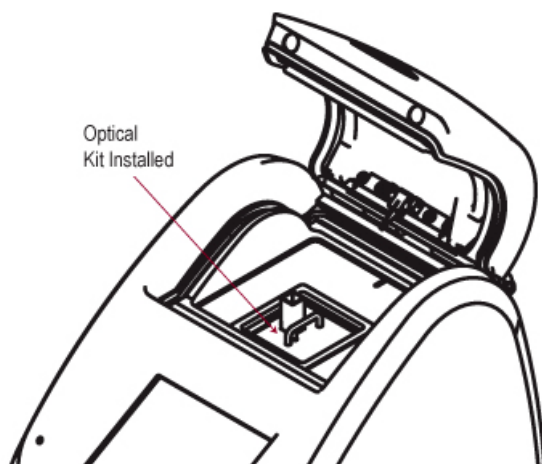


Figure 9.

Removal

1. Power OFF the Trilogy before removing the Optical Application Module
2. Grasp the handle and gently pull up to release the kit from the sample compartment.

Touch Screen Basics

See next page

Touch Screen Basics (Turbidity)

Home Screen

The "Home" screen appears after confirmation of the Optical Application Module. The "Home" screen provides orientation for the multiple functions of the Trilogy. From the "Home" screen, select "Calibrate," "Tools," "Mode," and "Help." The "Home" screen is also the measurement screen.

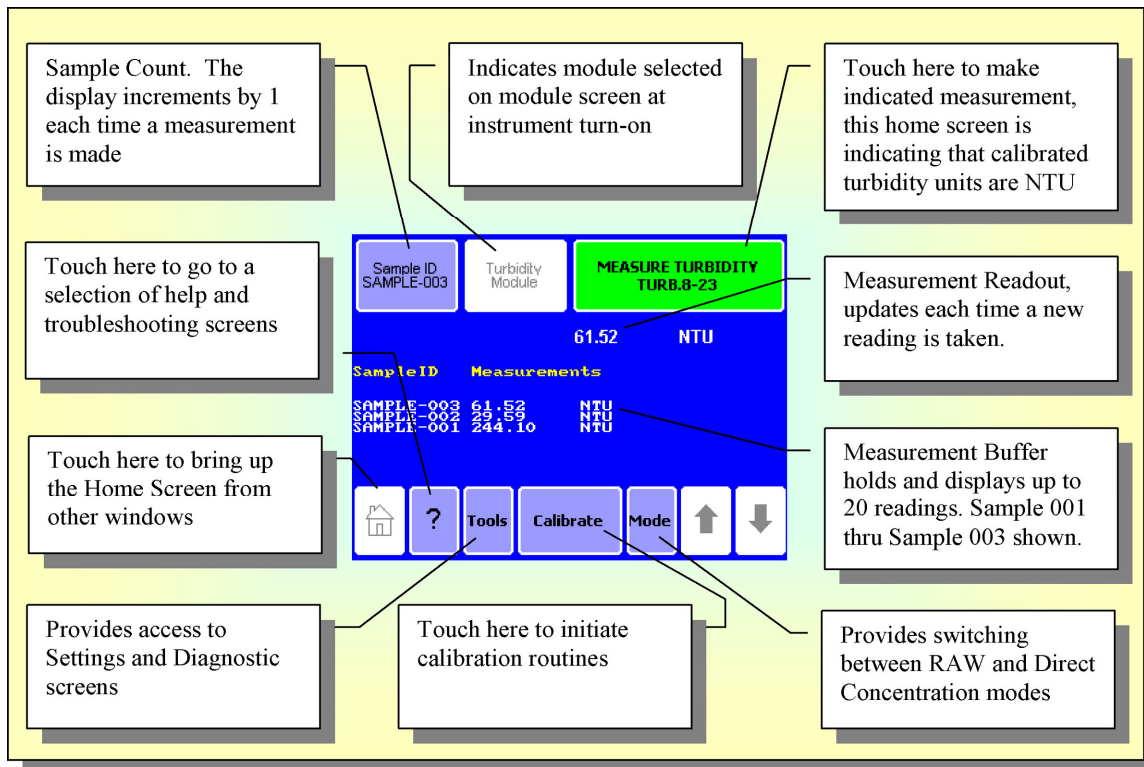


Figure 10: Home Screen When Using Turbidity Module

Calibration

Refer to Section V.

Measuring Samples

There are two measurement modes available on the Trilogy when using the Turbidity Module:

Raw Mode – No calibration required

Direct Concentration Mode – Calibration required – See Section V

Touch "**Mode**" on the Home Screen to select the measurement mode.

1. Raw Mode: The Raw Mode should be used for qualitative measurement, for example where measuring changes is required, rather than absolute concentration values. Readings are displayed in Nephelometric Turbidity Units (NTU).

2. Direct Concentration Mode: The Direct Concentration mode makes absolute measurements based on a calibration, see Section V for the Calibration Procedure.

Use polystyrene cuvettes for measuring turbidity.

1. Open the lid of the Trilogy and insert the cuvette. Close the lid.
2. Touch "Sample ID" to name your sample (optional).
3. Using the keypad, enter the sample name into the name field. Touch "Save" to save the sample ID.
4. Touch "Measure Turbidity" to commence measurement. The Trilogy will measure the sample for 6 seconds and report the average reading for the sample.

The Trilogy reports data on the "Home" screen and displays the results for the most recent 20 measurements. Use the arrow keys to scroll through the most recent measurements. The data automatically exports to a printer or PC when properly connected, (see Section VII). Please note the Trilogy does not store more than 20 measurements at one time. If more than 20 readings are taken, the oldest reading will be overwritten. Measurements are not stored between power cycles.

Tools (Turbidity)

Touch the "Tools" key to access "Settings."

Tools - Settings

View Cal Details

Touch "View Cal Details" to see information on the current calibration for Direct Concentration Mode. "View Cal Details" specifically provides information on the raw fluorescence for each standard and the blank as well as the unit of measure and the Optical Application Module.

Continuous Sampling

The Continuous Sampling feature enables repeat measurements at user-defined intervals.

1. Touch "Continuous Sampling" and turn the feature ON. Highlight the frequency of measurement and the number of total measurements. The maximum number of total measurements is 9999.
2. Touch "OK" to return to the "Home" screen.
3. Connect the Trilogy to a printer or a PC to collect the data. Touching the screen repeatedly causes an early-abort of Continuous Sampling measurements.

Measurement Tip:

On the Home Screen, touch **Settings** then touch the **Lid Start** key to turn the feature ON. When the Lid Start feature is ON, measurement begins as soon as the lid closes. The lid start feature allows for immediate measurement and eliminates the need to touch the "Measure" key. Also, the touch screen does not hibernate when Lid Start is ON.

Return to the **Lid Start** key under the **Settings** menu to turn the feature OFF.

V. Calibration Overview

A. Why Calibrate

The Trilogy calibration procedure calculates the fluorescent signal to your units of measure. Once calibrated, the Trilogy can give you concentration readings directly, saving you from having to perform any calculations.

B. When to Calibrate

- For greatest accuracy, calibrate before running a new batch of samples.
- Recalibrate if the ambient temperature changes by +/- 5°C.
- Recalibrate after changing to a different optical module, or if you make measurements on a new analyte.
- Verify the need to calibrate by reading a stable, known concentration standard immediately after calibration and again every few hours to see if readings have changed significantly. Recalibrate when the accuracy becomes unacceptable for your study.

C. Trilogy Calibration Options, (Fluorescence and Turbidity)

There are two measurement modes available on the Trilogy when using either the Fluorescence or Turbidity Modules, (see section K for Absorbance Module Calibration Procedure):

Raw Fluorescence Mode – No calibration required

Direct Concentration Mode – Calibration required

1. Raw Fluorescence Mode: The Raw Fluorescence Mode should be used for qualitative measurement, for example where measuring changes is required, rather than absolute concentration values. Readings are displayed in Relative Fluorescence Units (RFU).

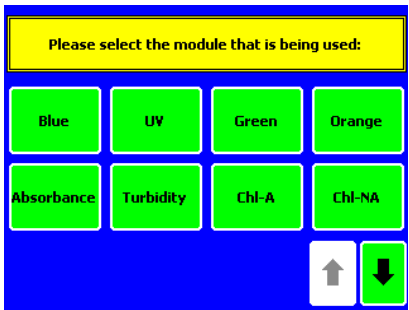
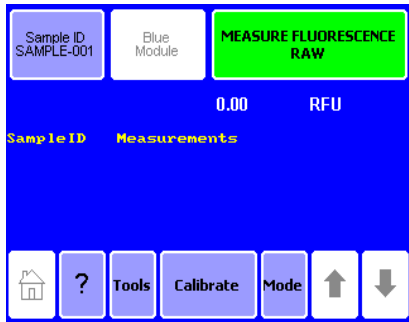
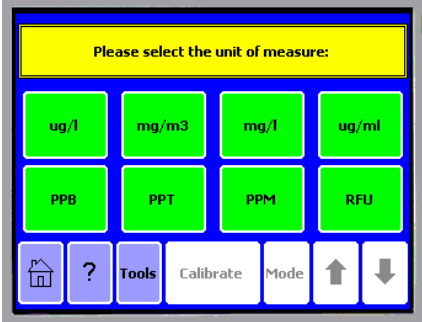
2. Direct Concentration Calibration: Direct Concentrations can be calibrated by using single or multi-point calibrations. In multi-point calibrations, up to five standards and a blank can be read generating a calibration curve for superior accuracy. The software uses these points to calculate direct concentrations. The Trilogy will display the actual concentration of your samples in units that were chosen during calibration.

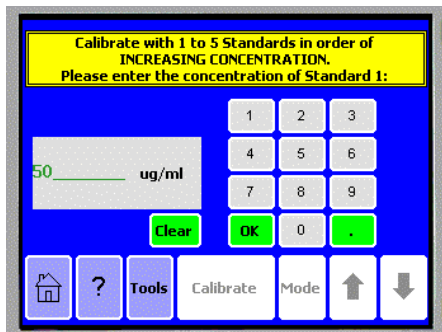
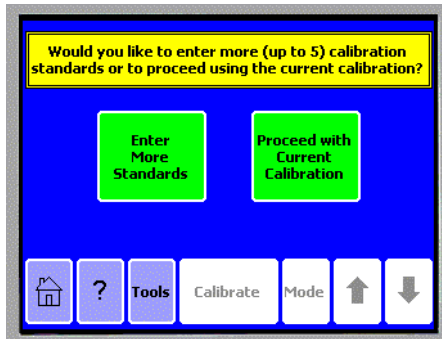
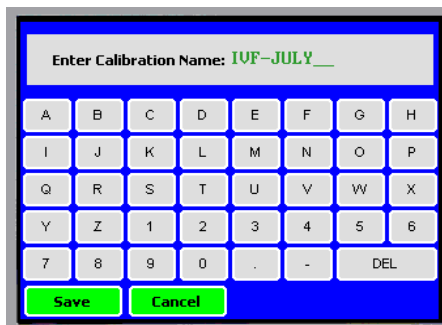
D. Trilogy Calibration Procedures

- 1. Raw Fluorescence Mode:** A calibration is not necessary to measure fluorescence with the Trilogy. Simply use the Raw Fluorescence Mode to obtain the fluorescent value of a sample in Relative Fluorescence Units (RFU). Use the standard curve to determine the concentration of the analyte in the samples. The Trilogy does not manipulate the data while operating in the Raw Fluorescence Mode. It is not necessary to zero the Trilogy for use in the Raw Fluorescence Mode, however a blank sample should be run to determine background fluorescence. A solid secondary standard may be used to verify instrument stability and function (see section J).
- 2. Direct Calibration Mode:** The Direct Concentration Mode requires a calibration with one blank solution and at least one standard solution. The following procedure applies to all the fluorescence modules with the exception of the Chl Acidification and Non-Acidification modules. (There are separate procedures for these two exceptions). The procedure requires the use of at least one calibration standard, (Chlorophyll a , Rhodamine WT, etc.). Up to 5 standard solutions can be used for a Multi Point Calibration. Calibrations can be given a name and stored for future use.

E. “Direct Calibration” Procedure – a) Fluorescence and Turbidity Modules, Single Point and Multi Point Cal

The following procedure applies to Trilogy and the Fluorescence or Turbidity Modules. See page 20 for procedures to calibrate the Chl Acidification and Non-Acidification Modules.

Instructions	Sample Screen
a) Turn on the Trilogy	
b) Select the module/application to be calibrated and confirm by touching “OK”. Note: Refer to the module label to confirm which module is in use.	
c) On the home screen, touch “Calibrate” to begin a calibration sequence	
d) Select “Run New Calibration”	
e) Select the unit of measurement	
f) Insert the calibration “blank” and touch “OK”	

<p>g) Enter the concentration for the first Standard. If using the Turner Designs Chlorophyll a Standard, this will be the concentration data supplied with the Standard. If doing multi point calibrations, be sure to use Standards in order of increasing concentration.</p>	
<p>h) Follow the screen prompt indicating that the standard should be inserted, touch OK</p>	
<p>i) After the calibration is complete, either select “Proceed with Current Calibration” or select “Enter More Standards”, in which case, return to “g” above.</p>	
<p>j) Save the calibration for future use (optional).</p>	
<p>k) Subsequent readings in the Direct Concentration mode reflect the actual concentration of the analyte.</p>	
<p>l) Confirm successful completion of the calibration by measuring the same Standard. The displayed concentration should equal the value used in step “g” above. Optionally, the Solid Secondary Standard could now be adjusted to give the same reading for future calibrations, see Section K.</p>	<p>This completes the “Direct Calibration” Procedure for the Fluorescence and Turbidity Modules.</p>

F. Extracted Chlorophyll Measurements with the Chl a – Acidification Fluorescent Module

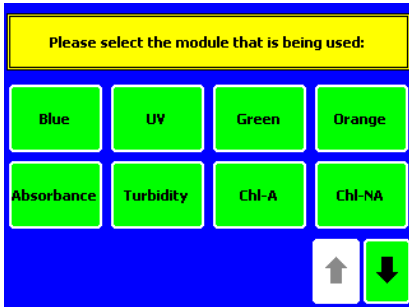
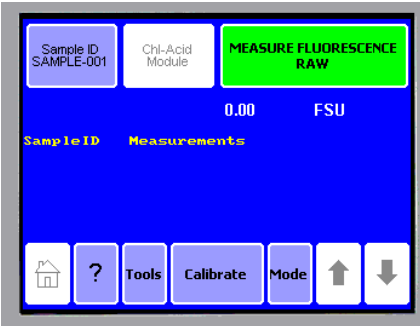
EPA Method 445.0 is a standard method for measuring extracted chlorophyll *a* and pheophytin *a* in marine and fresh water algae by fluorescence. It requires extraction with 90% acetone, measurements before and after acidification and some fairly simple calculations to arrive at the chlorophyll *a* and pheophytin concentrations (see appendix F). Method 445.0 is detailed and straightforward. (if high concentrations of pure chlorophyll *b* are present, see the next paragraph)

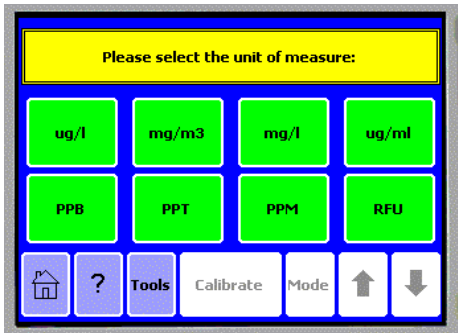
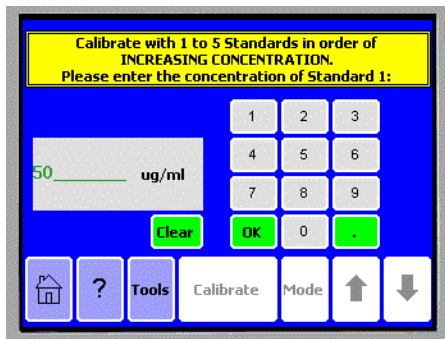
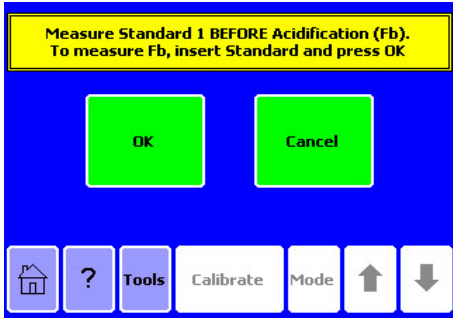
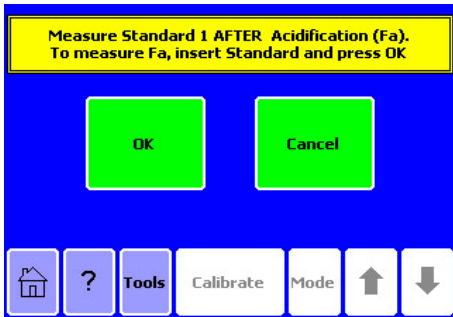
A known concentration of pure chlorophyll *a* (as a standard) is required at least the first time you calibrate Trilogy. For greatest accuracy however, we recommend that you periodically (once every few months) use a known concentration of pure chlorophyll *a* in 90% acetone to recalibrate your instrument. (Liquid Primary and Solid Secondary Chlorophyll *a* standards are available from Turner Designs).

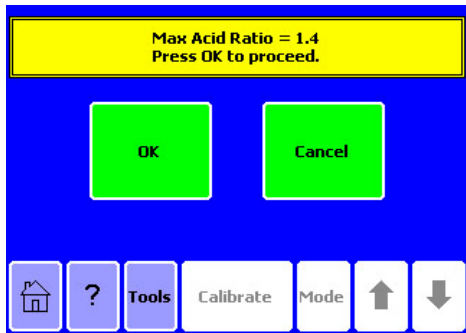
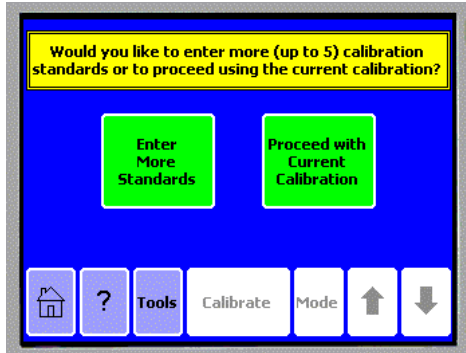
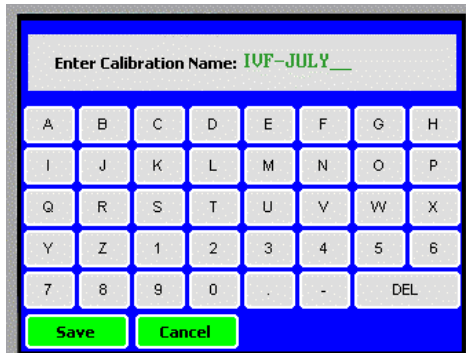
G. Calibrating and Displaying Corrected Readings for the Chl a Acidification and Non-Acidification Modules

The Trilogy firmware includes the correction of displayed chlorophyll readings to include the filtered and solvent volumes used during the calibration and measurement cycles. Be sure to touch either “**Chl A**” or “**Chl NA**” selections in the module selection screen, so that the appropriate corrections are applied to the measured data. This results in displayed readings that correspond to the actual sample concentration.

H. “Direct Calibration” Procedure – b) Extraction, Acidification, Single Point and Multi Point

Instructions	Sample Screen
a) Turn on the Trilogy	
b) Touch “ Chl – A ” to select the Chlorophyll <i>a</i> – Acidification module and calibration firmware, and confirm by touching “ OK ”	
c) On the home screen, touch “ Calibrate ” to begin a calibration sequence	

d) Select “Run New Calibration”	
e) Select the unit of measurement	
f) Insert calibration “blank” and touch “OK”	
g) Enter the concentration for the first Standard. If using the Turner Designs Chlorophyll a Standard, this will be the concentration data supplied with the Standard. If doing multi point calibrations, be sure to use Standards in order of increasing concentration.	
h) Follow the screen prompt indicating that the Standard before Acidification (Fb) should be inserted. Insert sample and touch “OK” .	
i) Now insert the Standard after Acidification and touch “OK” . The (Fa) value will be measured and the ratio of the two readings will be displayed as seen in the next step.	

<p>j) If the ratio is within the required range, touch “OK”. The ratio will be stored in the Trilogy for use in the EPA Method 445.0 for measurement of chlorophyll <i>a</i> and pheophytin <i>a</i> in water.</p>	
<p>k) After the calibration is complete, either select “Proceed with Current Calibration” or select “Enter More Standards”, in which case, return to “g” above.</p>	
<p>l) Save the calibration for future use (optional).</p>	
<p>m) It is recommended that the Solid Standard is now measured and the displayed value noted to enable a quick calibration check prior to later use.</p>	
<p>This completes the “Direct Calibration” Procedure – b) Extraction, Acidification, Single Point and Multi Point</p>	

I. “Direct Calibration” Procedure – c) Extraction, Non-Acidification, Single Point and Multi Point

The Welschmeyer method is a new, simplified way to measure chlorophyll *a* without the need for acidification. It accurately measures chlorophyll *a* even in the presence of chlorophyll *b* and pheophytin. However, you **cannot** obtain a pheophytin *a* measurement with this procedure. Using this method, you extract your samples according to EPA Method 445.0, but skip the acidification step.

You still need to calibrate the instrument the first time using a known concentration of pure chlorophyll *a* in 90% acetone.

The calibration procedure for the Chlorophyll Non-Acidification follows the same steps as for the “Direct Calibration” mode, see Section E, however the measurement procedure will prompt for the filtered and solvent volumes.

J. Using the Secondary Standard

The following section will describe how to use the Solid Secondary Standard, P/No. 8000-952, with most of the Trilogy fluorescence optical modules, (Chlorophyll *a*, Rhodamine WT, etc., but not ammonium). The two main benefits of using the Solid Secondary Standard are:

- 1) It can be used in place of a primary liquid standard once a correlation between a primary standard and the solid standard has been established.
- 2) It can be used to check the fluorometer stability, and/or check for loss in sensitivity resulting from instrument/optical module problems.



Figure 11. The Adjustable Secondary Standard includes a fluorescent rod that provides an extremely stable signal.

The Solid Secondary Standard provides a very stable fluorescent signal. It has an adjustment screw so that you can tune the Solid Standard to provide a signal to match a specific sample. It should be noted that each Solid Standard/Fluorometer relationship is unique. This means that a given Solid Standard can not be used to calibrate multiple fluorometers or modules for identical readings of a given solution.

K. Using the Solid Secondary Standard for Chlorophyll Applications

1. To establish a correlation between a known chlorophyll concentration and the fluorometer reading, measure a sample containing algae and note the fluorometer reading.
2. Insert the Solid Standard in the Optical Module, and adjust the Solid Standard to produce the same reading on the fluorometer as in step 1, (turning the Secondary Standard adjustment screw clockwise produces a lower signal).
3. Next, perform a chlorophyll extraction using the Trilogy Laboratory Fluorometer, Spectrophotometer or HPLC to determine the actual chlorophyll *a* concentration in the sample¹. This will provide the correlation between the solid standard and the actual chlorophyll *a* concentration.
4. Now, at any time, the Solid Standard can be used to check/establish a new correlation between a known equivalent concentration and the current Trilogy reading.

¹ Information on doing a chlorophyll extraction can be found on the Turner Designs web site at this URL: http://www.turnerdesigns.com/t2/doc/appnotes/998_9000.html

L. Use of the Solid Secondary Standard for Dye Applications:

The Solid Secondary Standard accessory can also be used to check the fluorometer stability for making dye concentration measurements. If necessary, the Solid Standard can be used to establish a new correlation factor without the need to use a calibration solution each time.

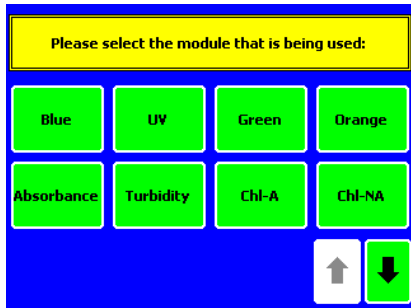
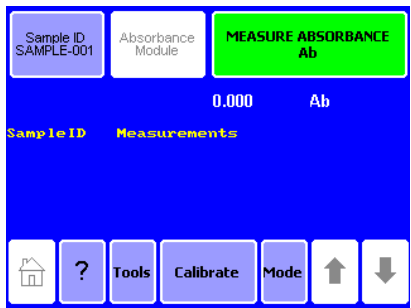
1. To use the Solid Standard to establish a correlation between a known dye concentration and the fluorometer reading, measure a dye solution of known concentration, say 50 ppb, and note the Trilogy reading.
2. Place the Solid Standard in the Optical Module, and turn the adjustment screw to produce the same displayed concentration as in step 1, (turning the secondary standard adjustment screw clockwise reduces the displayed concentration).

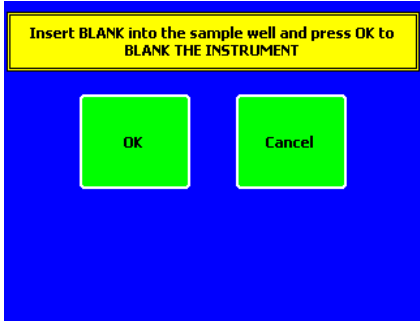
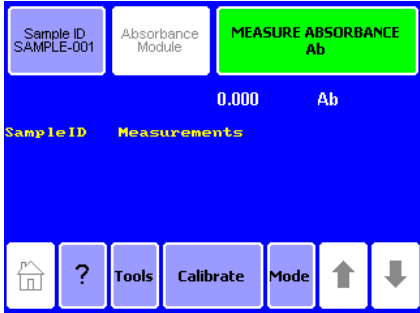
Comprehensive information on dye trace measurements can be found at the following Turner Designs URL:

<http://www.turnerdesigns.com/t2/doc/appnotes/main.html#fluorescent>

M. Trilogy Calibration Procedure, (Absorbance Module)

Calibrate the Trilogy after powering up, and after changing filters. For best results, calibrate the Trilogy and Absorbance Optical Module immediately before reading a series of samples.

Instructions	Sample Screen
a) Turn on the Trilogy	
b) Touch " Absorbance " to select the Absorbance Application Module and calibration firmware, and confirm by touching " OK "	
c) On the home screen, touch " Calibrate " to update the blank	

<p>d) Insert a sample cuvette containing your blank sample and touch “OK” to complete the calibration update.</p>	
<p>e) When the blanking is complete, the display will revert to the Absorbance Home/ Measurement Screen.</p>	
<p>f) This completes the Absorbance calibration procedure, and the Trilogy is now ready to make Absorbance measurements.</p>	

VI. Touch Screen Basics

Touch Screen

The touch screen provides a user-friendly method to operate the Trilogy. The touch screen is sensitive to the light pressure of a fingertip. It is not necessary to use a stylus. After 20 minutes without activity or user stimulation, the touch screen hibernates. Lightly touch the screen once to reactivate. To select a function, touch the button corresponding to the function once.

Tools

Touch the "Tools" key to access "Settings" and "Diagnostics."

Settings

Contrast

Touch the "Contrast" key to increase or decrease the brightness of the touch screen and enhance visibility. The arrows increase or decrease contrast. Touch the "Home" key to save the adjustment and return to the "Home" screen.

Reset

The "Reset" button restarts the Trilogy. Normal operation does not require this feature. The Reset feature erases the data displayed on the "Home" screen.

Lid Start

Touch the "Lid Start" key to turn the feature ON. While the Lid Start feature is ON, measurement begins as soon as the lid closes and the touch screen does not hibernate. The Lid Start feature allows for immediate measurement and eliminates the need to touch the "Measure" key. Return to the Lid Start key under the "Settings" menu to turn the feature OFF.

Diagnostics

Touch Screen Calibration

The "Diagnostics" menu contains a method for screen calibration. Although the touch screen is calibrated at the factory, it may need re-calibration over time.

Device Configuration

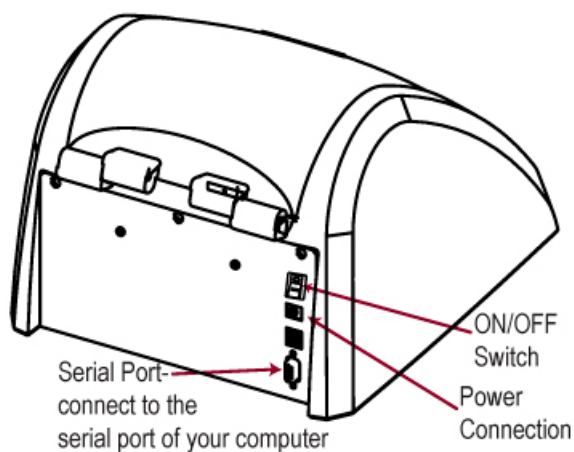
The "Device Configuration" key contains useful information on firmware revisions and instrument setup.

General

Please note the Trilogy does not store more than 20 measurements at one time. Measurements are not stored between power cycles.

VII. Systems Connections

Establish a connection between the Trilogy and a PC or a printer to export data. Connect the 9-pin RS-232 serial cable between the Trilogy and the PC or printer. The male 9-pin connector attaches to the Trilogy and the female connector attaches to the PC.



Serial Output Functional Check

To perform a functional check on the Trilogy Serial Output, complete the above for the Trilogy, and then complete the following steps, (note that the Serial Interface Software is not required):

1. Connect the Trilogy Serial Port to the PC Serial Port.
2. To receive ASCII data from the Trilogy, open a HyperTerminal program, or some other appropriate data logging software on the PC.
3. Open a connection in HyperTerminal with the following COM Port settings:

Baud Rate:	9600
Data Bits:	8
Parity:	None
Stop Bits:	1
Flow Control:	None

4. Make a measurement with the Trilogy
5. At the completion of the measurement, the concentration value will be displayed on the Trilogy display window, and also displayed in the HyperTerminal window.

This completes the Functional Check of the Trilogy Serial output. If you successfully completed these steps, the Trilogy Serial Output is functioning. If you were not able to complete these steps, refer to the troubleshooting section of this manual.

VIII. Spreadsheet Interface Software Installation

Install the Spreadsheet Interface Software (SIS) to send data to an Excel spreadsheet. The Spreadsheet Interface Software requires a PC loaded with Windows '98 or higher, an available serial port, and Excel. Insert the software CD-ROM into the CD-ROM drive of the PC to initiate the installation program. After the installation is complete, an icon for the SIS appears on the PC desktop and in the "Programs" menu.

(Make sure no other program on the PC is already using COM 1).

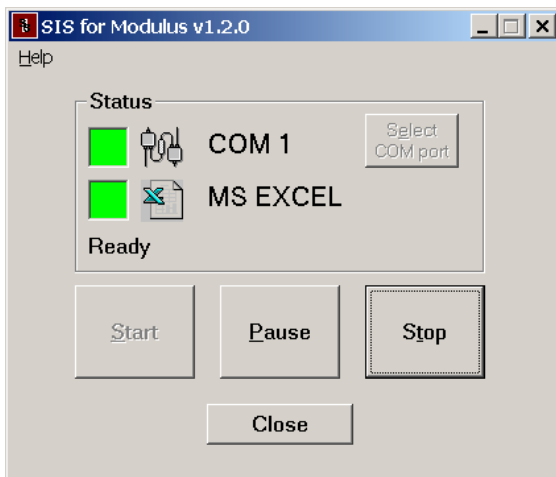


Figure12:
Spreadsheet Interface Software status window showing communications are set up on COM 1, and that the data will be displayed in an EXCEL spreadsheet

Excel Spreadsheet Example

Turner Designs

SAMPLE-001	426.73 RFU
SAMPLE-002	24.57 RFU
SAMPLE-003	35.49 RFU
SAMPLE-004	2.56 RFU

Appendix A - Troubleshooting

Fluorescence Troubleshooting

Symptom	Possible Solution
Bad calibration error message	A bad calibration error message may occur if the blank is brighter than the standard. Compare the reading of the standard and the blank in the Raw mode.
Erratic reading	When direct fluorescence readings do not produce expected values, review the standard value entered during the calibration. The number of the standard value should correspond to the actual concentration of the standard.
Negative values	After calibration, the blank value is automatically subtracted from subsequent readings. A negative reading can occur if a sample reading is less than the blank.
Low readings	Check the excitation and emission wavelengths of the analyte against the specifications of the Fluorescence Optical Application Module in use. Different analytes require different Optical Application Modules.
High background	A wet cuvette or spill could contaminate the cuvette holder and increase the background signal. Carefully clean the cuvette holder with 70% ethanol

Absorbance Troubleshooting

Symptom	Possible Solution
Non-linear response	Many absorbance assays do not produce a linear response but instead produce a sigmoidal or pseudo-sigmoidal response. Refer to the Application Note for the assay for more information.
Low readings	Check the filter installed in the Absorbance Module and make sure it is the correct filter for the assay. View the Calibration details from the Tools menu
Bad Calibration Error Message	Install the proper filter and use the ultrapure water in a clean cuvette to update the zero. Check the Calibration details from the Tools menu.

Turbidity Troubleshooting

Symptom	Possible Solution
Trilogy readings do not agree with other Turbidity meters	Calibrate both meters with the same calibration standard solution. If meters still display significantly different readings, it may be that the second turbidity meter does not make an IR measurement, and the sample contains interference colors.
The turbidity readings change each time a reading is taken	This is normal. Particles in a liquid sample do not remain in the same position, and these position changes affect the scattering of the light, and therefore the turbidity reading.
My turbidity readings seem to be different when I re-calibrated with a new primary standard.	Formazine standards form the basis of all turbidity measurements and they are very susceptible to aging. ISO 7027 recommendation specifies that the 4,000 NTU Formazine solution can be kept for only 4 weeks. For consistent readings calibrate with current standards.

Spreadsheet Interface Software Troubleshooting

Symptom	Possible Cause	Possible Solution
Excel does not open	Excel is not installed on the PC.	Make sure Excel is installed on your PC.
Excel does not open.	The software cannot find Excel.	Open Excel from the Programs Menu on the PC then open the spreadsheet interface software.
Both green lights are on, but data does not appear in Excel	Wrong COM port selected.	Click "STOP" then click on the "COM" button to change the COM port.
	Fluorometer not connected to PC.	Check the RS-232 connection between the Trilogy and the PC.
New data does not report to Excel	There is an editing process occurring within an Excel cell.	Wait until all the data is collected before editing the Excel spreadsheet.

The software does not install	The PC allows only administrators to install new software.	Log in as Administrator for the PC, then install the software or contact your IT support desk.
The software does not open	The software was not installed properly.	Log in as Administrator. Remove the software and re-install.

Appendix B - Warranty and Obtaining Service

Warranty

Turner Designs warrants the Trilogy Fluorometer and accessories to be free from defects in materials and workmanship under normal use and service for a period of 12 months from the date of shipment from Turner Designs with the following restrictions:

The instrument and accessories must be installed, powered and operated in compliance with the directions in this Trilogy User's Manual and directions accompanying the accessories.

Damage incurred in shipping is not covered

Warranty Service

To obtain service during the warranty period, the owner shall take the following steps:

Write or call the Turner Designs Technical Support Department and describe as precisely as possible the nature of the problem.

Phone: 1 (877) 316-8049

E-Mail: support@turnerdesigns.com

Carry out minor adjustments or tests as suggested by the Technical Support Department.

If proper performance is not obtained, ship the instrument, prepaid, to Turner Designs, with a statement of shipping charges. The instrument will be repaired and returned free of charge, along with a check to cover shipping charges, for all customers in the contiguous continental United States.

For customers outside of the contiguous continental United States, and who have purchased our equipment from one of our authorized distributors, contact the distributor. If you have purchased direct, contact us. We will repair the instrument at no charge: shipment; documentation; etc; charges will be billed at cost to the customer.

NOTE! The instrument or accessories should not be returned without first contacting Turner Designs. Prior correspondence, including an RMA number is needed:

- a. To ensure that the problem is not a customer solvable one, easily handled in your laboratory, saving the end-user time and money.
- b. To specifically determine the nature of the problem, so that repair can be rapid, with particular attention paid to the defect you have noted.

Out of Warranty Service

Follow the same steps as for Warranty Service. Our service department is happy to assist you by telephone or correspondence at no charge. Repair service will be billed at a flat rate. Your invoice will include freight charges.

Address for Shipment:

Turner Designs
845 W. Maude Ave.
Sunnyvale, CA 94085
USA

Telephone: 408-749-0994
Toll-Free: 877-316-8049 (US & Canada)
Fax: 408-749-0998

Appendix C – Specifications

Fluorescence Module Performance

Minimum Detection Limit:

Chlorophyll a, 0.02 ug/L
Rhodamine WT 0.02 ppb

Dynamic Range:

0-1000 ppb RWT

Linearity:

0.99R²

Turbidity Module Performance

Minimum Detection Limit:

0.05 NTU

Dynamic Range:

0 – 1,000 NTU

Linearity:

0.99R²

Absorbance Module Performance

Photometric Measuring Range:

0.0 – 4.0A

Photometric Accuracy:

+/- 0.7%

Photometric Precision:

<0.5% at 1A

General Specifications

Sample Adaptors

Accommodates 10 x 10 mm square plastic cuvettes, 12 x 75 mm round tubes, and 12 x 35 mm round vials.

Readout:

Direct Concentration (ug/L, ppb, etc.) or Raw Fluorescence

Calibration:

One to Five point calibration with up to 18 calibrations stored

Light Source & Detector:

LED and Photodiode

Blank:

Reads and subtracts blank

Data Output:

100% ASCII format through a 9 pin RS-232 serial cable at 9600 baud

PC Operating System (optional if connected to PC):

Windows 98® or later

Power:

100 to 240VAC Universal Power Supply included, Output 12VDC 0.84A Max

Dimensions:

12.92"D x 10.44"W x 8.42"H
(32.82 cm D x 26.52 cm W x 21.39 cm H)

Operating Temperature:

60 – 105 °F (15 - 40 °C)

Weight:

8.1 lbs (3.65 kg)

Humidity:

75% RH maximum

Warranty:

One year

Application Module Filter Wavelengths

Fluorescence Modules			
Application	Part Number	Excitation Wavelength	Emission Wavelength
Chlorophyll a, Acidification	7200-040	485 nm	685/50 nm
Chlorophyll a, non-acidification	7200-046	485 nm	685/10 nm
Chlorophyll a, <i>In Vivo</i>	7200-043	485 nm	685/50 nm
Phycocyanin	7200-044	600 nm	640 nm
Rhodamine WT & Phycoerythrin	7200-042	550 nm	610 nm
Fluorescein	7200-048	485 nm	540 nm
CDOM & Ammonium	7200-041	365 nm	430 nm
Turbidity Module			
Application	Part Number	Wavelength	
Turbidity	7200-060	850 nm	
Absorbance Module & Filter Kits			
Description		Part Number	
Absorbance Module		7200-050	
560/10 nm Filter Paddle		7200-051	
600/10 nm Filter Paddle		7200-052	
750/10 nm Filter Paddle		7200-053	
Contact us for other applications or wavelengths			

Specifications subject to change without notice

Included Accessories

10 each 10 x 10 mm Methacrylate Cuvettes
5 each 12 x 35 mm Screw Top Glass Vials
50-Well Storage Box
Solid Secondary Standard
Power Supply
12 mm Round Vial Adaptor
CD with User's Guide and Spreadsheet
Interface Software

Appendix D – Principles of Fluorescence

Principles of Fluorescence

Fluorescence is a physical property of certain atoms and molecules. It is a molecule's ability to absorb light energy at one wavelength, then instantaneously re-emit light energy of another, usually longer, wavelength. Each compound that fluoresces has a characteristic excitation wavelength, (the wavelength of light that it absorbs) and a characteristic emission wavelength, (the wavelength of light that it emits when the molecules relax and return to their ground state). These excitation and emission wavelengths, (or spectra), are often referred to as the compound's fluorescence signature. Figure 13 shows the key components of a filter fluorometer.

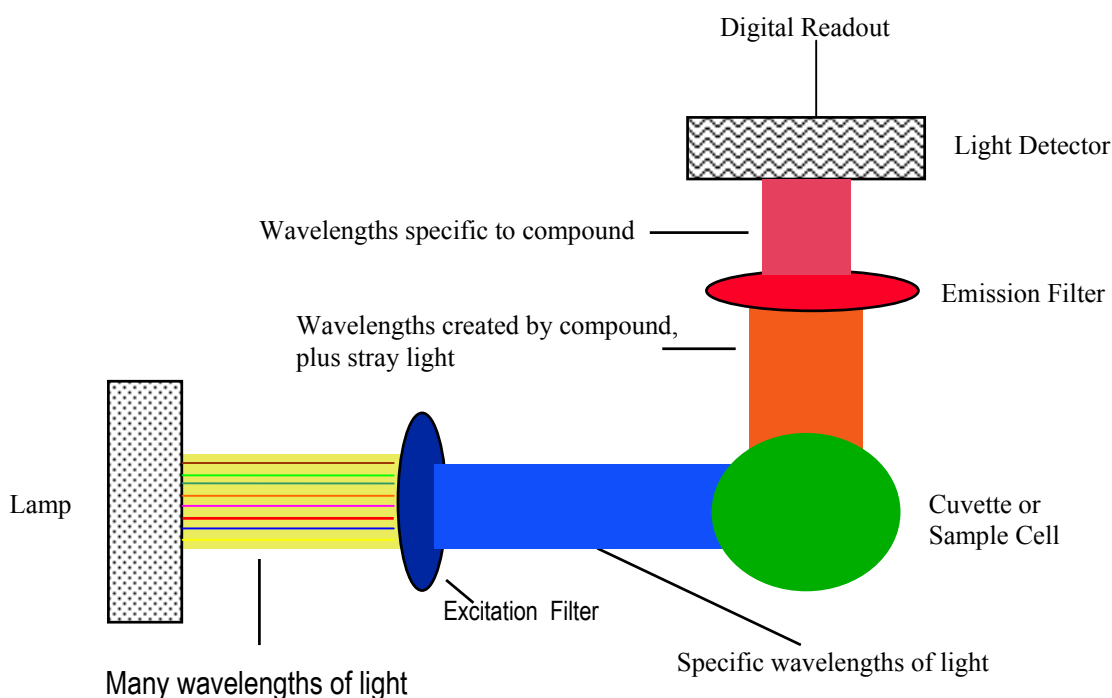


Figure 13

Lamp: The lamp or light emitting diode provides the light energy that "excites" the compound of interest. The lamp actually provides a broader range of light than that which excites the compound. This broad light range is illustrated by the "many wavelengths of light" shown in Figure 13.

Appendix E – Recommended Measurement Practices

Linear Range and Quenching

The linear range is the concentration range in which the Trilogy output is directly proportional to the concentration of the fluorophore. The linear range begins with the smallest detectable concentration, and spans to an upper limit (concentration) that is dependent upon: the properties of the fluorescent material; the filters used; and the path length.

A non-linear relationship is seen at very high concentrations where the fluorescence signal does not increase at a constant rate in comparison to the change in concentration, see Figure 4 below. At even higher concentrations, the fluorescence signal will decrease even though the sample concentrations are continuing to increase. This effect is known as “signal quenching”.

Linearity may be checked by diluting a sample 1:1, or some other convenient ratio. If the sample is still in the linear range, the reading will decrease in direct proportion to the dilution. If the reading does not decrease in direct proportion to the dilution, or if the reading increases, the sample is beyond the linear range of the fluorophore.

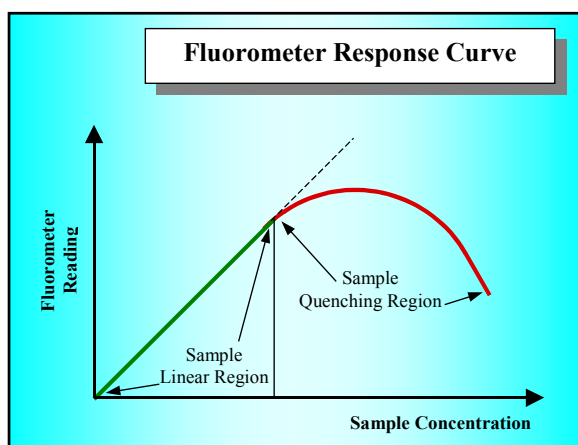


Figure 14
Graph showing Linear and Quenching Regions of the sample's response

Temperature Considerations

Fluorescence is temperature sensitive. As the temperature of the sample increases, the fluorescence decreases. For greatest accuracy, record the sample temperature and correct the sensor output for changes in temperature.

For further information on how temperature, light, water quality and the physiological state of the algal cells can all affect the measurement of chlorophyll a, please refer to the application section of Turner Designs' web site at the following URL:

<http://www.turnerdesigns.com/esupport/understanding.html>

Appendix F - Chlorophyll a Acidification and Non-Acidification calculations

When in direct concentration mode the following calculations will be use to calculate corrected chlorophyll a and pheophytin values for the acidification method and the corrected chlorophyll values for the non-acidification method.

Acidification Method

I. Variables stored during calibration phase of fluorometer

$C_{stand[1]}$	= Concentration of standard 1
F_{blank}	= Fluorescence of Blank value
$F_{stand[1],B}$	= Fluorescence of standard 1 before acidification
$F_{stand[1],A}$	= Fluorescence of standard 1 after acidification
F_m	= Acidification Ratio = $(F_{stand[1],B} - F_{blank}) / (F_{stand[1],A} - F_{blank})$

II. Variables required from the sample analysis phase

$F_{smp,B}$	= Fluorescence of sample before acidification
$F_{smp,A}$	= Fluorescence of sample after acidification
$V_{solvent}$	= Volume of solvent used to extract sample
V_{water}	= Volume of water filtered

III. Interpolation equation used in end calculation of chlorophyll a and pheophytin concentrations

$Interp_B$	= $C_{stand[1]} * (F_{smp,B} - F_{blank}) / (F_{stand[1],B} - F_{blank})$
$Interp_A$	= $C_{stand[1]} * (F_{smp,A} - F_{blank}) / (F_{stand[1],B} - F_{blank})$

IV. End calculation for corrected chlorophyll and pheophytin a

$$\text{Chlorophyll a concentration} = [F_m / (F_m - 1)] * (Interp_B - Interp_A) * (V_{solvent} / V_{water})$$

$$\text{Pheophytin a concentration} = [F_m / (F_m - 1)] * [(F_m * Interp_A) - Interp_B] * (V_{solvent} / V_{water})$$

Non Acidification Method

I. End calculation for corrected chlorophyll a

$$\text{Chlorophyll a concentration} = C_{stand[1]} * [(F_{smp} - F_{blank}) / (F_{stand[1]} - F_{blank})] * (V_{solvent} / V_{water})$$

Appendix G - *In vivo* fluorescence measurements using Trilogy

[In vivo chlorophyll a](#) analysis is the measurement of chlorophyll a fluorescence within a living cell. The advantage of this type of analysis is that it is quick and simple and does not require special sample preparation or extraction. It allows the user to measure a large number of samples in the field. However, without comparisons to extractive analysis, *in vivo* readings are qualitative in nature.

There are two modes for making *in vivo* measurements. The first utilizes the RAW fluorescence mode; the second requires calibrating to a secondary standard. No calibration is necessary for making *in vivo* measurements in the raw mode.

***In-vivo* measurements using the RAW mode:**

- 1) Insert the *in vivo* chlorophyll a Trilogy module Turner Part Number 7200-043
- 2) Turn on the Trilogy fluorometer using the switch located on the back panel
- 3) Select the "Blue" module. This is indicated on the module label under GUI selection.
- 4) Press "OK" after verifying that the module loaded matches your selection. The default mode loaded is "Raw Fluorescence".
- 5) Before measurements are made, it is advisable to filter a sample and measure the filtrate to obtain a blank or "Background" fluorescence reading for a given location
- 6) Thoroughly mix the sample by inverting or shaking to prevent settlement of algal cells and quickly insert sample
- 7) Close the lid and press the "Measure Fluorescence" button.
- 8) Subsequent readings will indicate relative changes in concentration levels. These readings will be presented in Relative Fluorescence Units of measure (RFU).

***In-vivo* Calibration Using Direct Concentration and a Secondary Standard:**

- 1) Insert the *in vivo* chlorophyll a Trilogy module Turner Part Number 7200-043
- 2) Turn on the Trilogy fluorometer using the switch located on the back panel
- 3) Select the "Blue" module. This is indicated on the module label under GUI selection.
- 4) Press "OK" after verifying that the module loaded matches your selection. The default mode loaded is "Raw Fluorescence".
- 5) Press "Calibrate"
- 6) Choose "Run New Calibration"
- 7) Choose the units to be displayed during measurement
- 8) Insert blank sample and press "OK"
- 9) When prompted enter a number, which will be correlated, to the sample's response. You may enter 1 to 5 standards of various concentration levels or calibration values.
 - If concentration of sample is known, enter the known value. This will set the fluorescence response equal to the concentration entered and will give semi-quantitative results in subsequent measurements (**example, 5(μ g/L) = 137.4(RFU)**)
 - If an arbitrary calibration value is entered, the fluorescence response of the sample will be correlated to the new calibration value and subsequent measurements will be scaled proportionally. These measurements will only give qualitative results (**example, 500(RFU) = 137.4(RFU)**)
- 10) Thoroughly mix sample by inverting or shaking to prevent settlement of algal cells and quickly insert sample
- 11) After inserting sample immediately press "OK" to measure
- 12) If running a multi-point calibration repeat step 9 through 11 for each calibrant

Blank Subtracting:

Blanks provide background fluorescence values of samples excluding the fluorophore of interest. Subtracting a blank sample from subsequent samples increases accuracy of fluorophore estimates. An accurate "blank" is typically a water sample that has been filtered through a GF/F or membrane filter in order to remove the algal cells but to still retain additional dissolved components.

Using the Solid Secondary Standard:

1. To establish a correlation between a known chlorophyll a concentration and the fluorometer reading, measure a sample containing algae and note the fluorometer reading
2. Insert the solid standard in the optical module and adjust the solid standard to produce the same reading on the fluorometer as in step 1, (Turning the solid standard screw clockwise produces a lower signal)
3. Next perform a chlorophyll a extraction using the Trilogy Laboratory Fluorometer to determine the actual chlorophyll a concentration in the sample. This will provide the correlation between the solid standard and the actual chlorophyll a concentration
4. The solid secondary standard can now be used to recalibrate and/or check the stability of the Trilogy fluorometer.

Turner Designs

845 W. Maude Ave.
Sunnyvale, CA 94085
Phone: (408) 749-0994
FAX: (408) 749-0998
www.turnerdesigns.com